

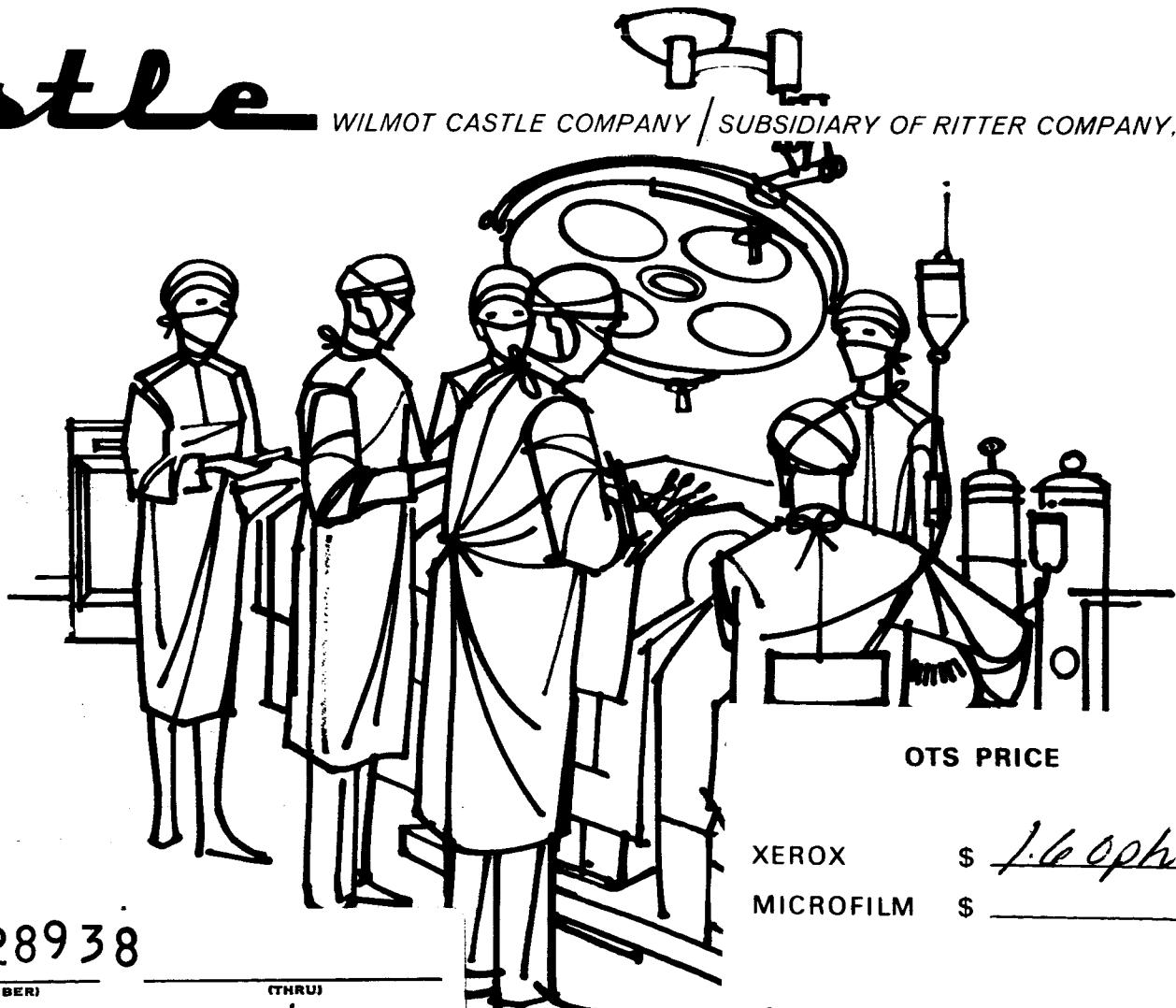
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STUDIES FOR STERILIZATION
OF
SPACE PROBE COMPONENTS

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PROGRESS REPORT NO. 1

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
CONTRACT NASw-879

SEPTEMBER 1st, 1963 - DECEMBER 1st, 1963

RESEARCH LABORATORIES
WILMOT CASTLE COMPANY
ROCHESTER, NEW YORK

STATUS OF REPORTS:

This Progress Report covers the research performed from September 1st, 1963 to December 1st, 1963 under NASA Contract NASw-879 by Wilmot Castle Company.

This report could not officially be issued until the contractual agreements were finalized. Since the contract has now been issued and is retroactive to September 1st, 1963, this Progress Report covers the first quarter.

The next Progress Report accordingly will be due March 1st, 1964, and will be issued thereon.

STATUS RESEARCH ACTIVITIES:

The current research activities have been concerned with the specific work objectives as modified by Dr. Freeman H. Quimby's letter of September 4th, 1963.

An unofficial report of results obtained was forwarded in the form of a letter to Dr. Carl W. Bruch (with copies to Dr. Freeman H. Quimby and Mr. Lawrence Hall) of the Biosciences Program Division on December 31st, 1963. This report includes the material reported in that communication.

CURRENT RESEARCH ACTIVITIES REPORTED HEREIN INCLUDE:

- I. Determination of times required to sterilize samples of activated carbon.
 - 1) with dry heat (see Table I)
 - 2) with moist heat i.e. saturated steam (see Table II)
- II. Determination of heat resistance of microorganisms collected from atmosphere. (see Table IV).

I. RESULTS AND DISCUSSION

- A. It would appear that carbon black, either with its natural contamination level or with spores added to it, in any of several manners, can be sterilized with dry heat in times less than those required for soil samples at the temperatures indicated (1) and not as resistant as Osowitz (2) has claimed.
- B. Experiments were also made using moist heat (i.e. steam under pressure) at 121°C for 15 and 30 min. The results are given in Table II for 0.05 g samples treated in metal capped tubes. It would appear that microorganisms in carbon black can also be effectively sterilized with conventional moist heat cycles.

TABLE I

OBSERVATIONS ON THE TIMES REQUIRED TO STERILIZE SAMPLES OF ACTIVATED CARBON
WITH DRY HEAT TREATMENTS

Material	Type and level of contamination	Observation*
Will Corp. brand (80 mesh)	natural microflora mesophilic aerobes 0.7 - 5 $\times 10^5$ per g.	Sterile after 1 hr. at 135 C in thioglycollate broth at 32C
	mesophilic anaerobes approx. 5 $\times 10^4$	Sterile after 3 hr. at 135C in trypticase soy broth at 32C
	thermophilic aerobes 2 $\times 10^4$ per g.	Sterile after 6 hr. at 135C in thioglycollate and trypticase soy broth at 55C**
	thermophilic anaerobes not detectable 0.01 g samples treated	
	Same as above 0.1 g samples treated	Sterile after 3 hr. at 135C in thioglycollate broth at 32C
		Sterile after 5 hrs. at 135C in trypticase soy broth at 32C
		Sterile after 6 hrs. at 135C in thioglycollate broth at 55C**
		Sterile after 12 hrs. at 135C in trypticase soy broth at 55C**
Merck brand #18351(N.F. Powder)	natural microflora level not established. 0.1 g samples treated	Sterile after 3 hrs. at 135C in thioglycollate broth at 32C Not sterile after 4 hrs. at 135C

TABLE I (cont'd)

Material	Type and level of contamination	Observation*
Will Corp. brand (80 mesh)	with spores of <u>Bacillus subtilis</u> var. <u>niger</u> at a level of 1×10^7 per 0.01 g. Spores added from a suspension then material dried in vacuum overnight. 0.01 g samples treated	Sterile after 1 hr. at 160C in trypticase soy broth at 32C
Will Corp. brand (80 mesh)	with spores of <u>Bacillus subtilis</u> var. <u>niger</u> at a level of 4.6×10^7 per 0.01 g Spores added from an acetone sus- pension and dried overnight. 0.01 g samples treated	Sterile after 8 hrs. at 135C in trypticase soy broth at 32C Sterile after 2 hrs. at 145C in trypticase soy broth at 32C Sterile after 1/2 hr. at 160C in trypticase soy broth at 32C
Will Corp. brand (80 mesh)	with spores of <u>Bacillus subtilis</u> var. <u>niger</u> at a level of 6×10^8 per 0.01 g. The organism was grown in the presence of carbon and harvested and dried with the carbon present. 0.01 g samples treated	Sterile after 8 hrs. at 135C in trypticase soy broth at 32C Sterile after 3 hrs. at 145C in trypticase soy broth at 32C Sterile after 3/4 hrs. at 160C in trypticase soy broth at 32C

* All samples incubated at least two weeks and subcultured at that time after treating in 150 x 16 mm screw-cap test tubes in dry heat units.

** Shortest time treated.

TABLE II

OBSERVATIONS ON THE TIMES REQUIRED TO STERILIZE SAMPLES OF ACTIVATED CARBON
WITH MOIST HEAT AT 121°C (15 lbs. PRESSURE)

<u>Test No. 1</u>		Treatment Time 15 min.			
Material: 0.05 g samples of <u>Merck Brand</u>		<u>Will Brand</u>			
Position of test tubes		Upright	Horizontal	Upright	Horizontal
No. showing growth	total number				
1/20*	20	0/20	2/20	0/20	1/20
Note: Results are of sterility test made by adding sterile thioglycollate broth to the tubes containing the samples after treatment and incubated at 32°C for at least two weeks.					
<u>Test No. 2</u>		Material: 0.05 g samples of Will Corp. Activated carbon		<u>Treatment Time</u>	
<u>Position of tubes</u>					
<u>15 min.</u>		<u>30 min.</u>			
horizontal		0/9*		1/9	
vertical		0/9		0/9	
vertical cap removed**		1/9		2/9	

* Results are given as number showing growth in trypticase soy broth after treatment and incubation at 32°C for at least two weeks.

** These samples could have been easily contaminated in handling.

II. RESULTS AND DISCUSSION

1. The filters chosen were of types which would not deteriorate in the temperature treatments anticipated (Table III).
2. The numbers and types of organisms being recovered consist of mold and bacteria. The assays reported are for the aerobic mesophilic bacterial spore populations which survived a heat shock treatment of 65 C for 30 min. Molds were recovered in non-heat shocked assays.
3. In reviewing some of the recent data on fallout from the atmosphere by the Fort Detrick group (3,4) it is obvious they were recovering most of the viable organisms whereas we are recovering only those which persist and resist the accompanied drying of organisms and aeration. Recent data indicated a build-up to a certain level of organisms and then it remains fairly constant when collected by filtration of the air. It should also be pointed out that the numbers being recovered on the filters are very low and hence one would expect a minimum of resistance of such populations. (Table IV).
4. Currently, sampling for longer periods of time are underway so as to obtain larger populations in order to determine the relative resistance of such to dry heat treatments.
5. In addition to the filtered samples, sterile stainless steel strips (2 in. x 0.25 in.) were left exposed in various areas of the laboratory for 24 hours. These strips yielded the following frequency distribution for organisms collected:

<u>Number of organisms</u>	<u>Number of strips</u>	<u>Results of Heat Treatments</u>
0-10	12	No strips produced growth after
10-50	12	1 hr. or greater treatment at
50-100	2	120C, when cultured in thioglycol-
100	3	late broth for at least two weeks
		incubation at 32 C

6. Further work is underway on the above areas. Articles which have been setting for long times and have collected a good layer of dust, (where maximum populations due to fallout of airborne organisms might be expected) are being assayed and evaluated as to the numbers of organisms and the cycles required to sterilize them.

TABLE III

CHARACTERISTICS OF MEMBRANE FILTERS EMPLOYED IN COLLECTING AIRBORNE OR
AEROSOLIZED MICROORGANISMS

<u>Manufacturer</u>	<u>Type</u>	<u>Material</u>	<u>Pore Size</u>	<u>Efficiency</u>
Millipore Filter Corp., Bedford, Massachusetts	HA	cellulose nitrate	0.45 μ ±0.2 μ	
Gelman Instrument Co., 106 N. Main St., Chelsea, Michigan	1) 6424 Versapor 2) type A glass fiber	epoxy membrane glass fibers	7.5 μ 99.6% of particles of 0.25 μ and above.	99 + % of particles of 0.25 μ and above.

The above filters were chosen because they are type which can tolerate (without charring, etc.) the temperature treatments anticipated.

TABLE IV

PRELIMINARY RESULTS OF THE RECOVERY OF MICROORGANISMS COLLECTED ON MEMBRANE FILTERS FROM VARIOUS AREAS AND THE HEAT RESISTANCE OF SUCH SAMPLES

Sample area	Sampling period	No. of samples	Average no. of bacteria per filter half ³	Results of heat treatment of organisms on filter halves.
1. Industrial Manufacturing area ¹	24 hr 48 hr 5 days	5 3 3	51 52 113	Sterile after 1 hr. at 120C
2. Microbiological laboratory	24 hr	12	10-100	Sterile after 1 hr. at 120C
3. Spore room ²	6 hr	3	910	Sterile after 1 hr. at 135C
4. Outdoor air	24 hr	16	30-1000	Sterile after 1 hr. at 120C

1 Normal dusty and dirty assembly areas.

2 Room in which spores of Bacillus subtilis var. niger were aerosolized due to laboratory operations. An average of 900-1000 spores of this organism were picked up per filter-half.

3 Aerobic mesophilic spore population as assayed after a heat shock of 65°C for 30 min. and plated on Plate Count Agar pour plates and incubated at 32 C.

REFERENCES

1. Koesterer, M. G. 1962 Sterilization of space probe components. Final report of NASA contract NASr-31. Wilmot Castle Company, Rochester, New York.
2. Osowitz, B. M. 1963 Personal communication article entitled "Of sterile origin" Trenton, New Jersey.
3. Portner, D. M., R. K. Hoffman, H. M. Decker, and C. R. Phillips 1963 Microbial contamination obtained on surfaces exposed to room air or touched by the human hand. Protection Branch Report of Test No. 1-64. July 22, 1963 Physical Defense Division, Fort Detrick, Frederick, Maryland.
4. Portner, D. M., R. K. Hoffman, H. M. Decker, and C. R. Phillips 1963 Sterilization of naturally contaminated metal surfaces with dry heat Protection Branch Report of Test No. 8-64 October 16, 1963 Physical Defense Division, Fort Detrick, Frederick, Maryland.

RESEARCH ACTIVITIES FOR SECOND QUARTER

1. Studies on the dry heat resistance of microorganisms:
 - a) Entrapped from air samples
 - b) Carried on sterile kaolin
 - c) In a nitrogen atmosphere.
2. Studies on the sterilization of components being initiated this next quarter include:
 - a) Components inoculated with heat resistant spores
 - b) Commercial components in the temperature range 100-135°C
 - c) Artificial or equivalent materials and/or components or objects which have accumulated natural contamination in an industrial manufacturing area.

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NASA Contract NASw-879